THIS THESIS IS SUBMITTED TO THE UNIVERISTY OF GHANA, LEGON IN PARTIAL FULFFILMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL IN MATERIALS SECIENCE AND ENGINEERING DEGREE

EXTRACTION OF BIOETHANOL FROM CORN HUSK

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DECEMBER, 2022

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DECLARATION

I hereby declare that this MPhil thesis Extraction of Bioethanol from corn Husk which has results of my original research was conducted in accordance with the University of Ghana's academic reputations and that either in whole or part has not been presented for another degree in the university.

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SUPERVISOR'S DECLARATION

I hereby declare that the preparation and presentation of this report was supervised in accordance with the guidelines on supervision of project reports laid by the University of Ghana.

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HEAD OF DEPARTMENT DECLARATION

I hereby declare that the preparation and presentation of this report was accepted in accordance with the guidelines on supervision of project reports laid by the University of Ghana.

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Signature:…….. Date: 5th January, 2023

DEDICATION

I dedicate this report to my wife and children, and to my younger brother at Agricultural Development Bank (ADB), Wa Branch, for their unflinching support throughout this research work. This report is also dedicated to my supervisor, Yaw Delali Bensah, PhD, for his sterling supervisory role in making this report a success.

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ABSTRACT

Well over a century, bioethanol, the most largely used liquid biofuel is produced by the fermentation of starch-and sucrose based-sources. However, it appears unattractive producing biofuel from food/feed sources which have the potential of hyping the competitiveness of their prices. The extraction of bioethanol from corn husk in this research seeks to explore more on the production of bioethanol using lignocellulosic biomass, by optimizing the treatment conditions to obtain the highest yield possible, and to analyze the distillate (i.e., the product) and evaluates its prospects for a potential biofuel. This research adopts a chemical extractive model (acid hydrolysis) by pretreating the corn husk with concentrated sulphuric acid, followed by the post-hydrolysis step where the feed acid concentration is reduced to a predetermined value. The approach adopted gave a high Brix value of 33°. A 14-day fermentation period of the hydrolysate is performed using a genetic modified variant of the microbial strain, *Saccharomyces cerevisiae*.

The raw powdered corn husk, the residual solid residue of the husk generated during hydrolysis, and the recovered bioethanol are subjected toa number of analytical and characterization techniques. These include X-ray powder diffraction (XRD) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, Gas chromatography-mass spectrometry (GC-MS) analysis, and Ultraviolet-visible light (UV-Vis)spectroscopy. The XRD pattern shows four matching crystallographic peaks for both the raw powdered husk and residual solid residue at different intensities producing an average of 38.50 % breakdown of the crystalline cellulose. FTIR analysis shows three frequencies of absorption peaks at 3332.00 nm, 2107.87 nm, and 1635.07 nm for an O - H, C= O, and C-H bending of an aromatic source, respectively. The GC-MS analysis identifies about 41 different chemical components present in the distillate, comprising organic acids, inorganic compounds, and organic salts. The chemical component with the highest percent compositionis n-Hexadecanoic acid, making 26 % of peak area. UV-Vis spectroscopy analysis qualitatively identifies ethanol in the distillate by comparing the spectrum of the distillate to the spectrum of standard grade ethanol at 99.8 wt % purity. The both graphs show absorption in the range 220 nm - 280 nm. Further UV-Vis

spectroscopy analysis characterizes the concentration of ethanol in the distillate at 10.0875 v/v %.

Key words: Bioethanol, polysaccharides, lignocellulosic, hydrolysis, fermentation, distillate, Characterization, spectroscopy, crystallographic.

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LIST OF ACRONYMS

1. MMDAs -------Metropolitan Municipal and District Assemblies
2. COP21 ---------United Nations sponsored Conference of Parties
3. GHG---------- Green House Gas
4. G1---------- First Generation
5. G2---------- Second Generation
6. CBP---------- Consolidated Bioprocessing
7. CBMs ---------Catalytic and Carbohydrate-binding Modules
8. SSF ----------Simultaneous Saccharification and Fermentation
9. SHF---------- Separate Hydrolysis and Fermentation
10. SSCF ----------Simultaneous Saccharification and Cofermentation
11. SPORL--------- Sulphite Pretreatment of Recalcitrant Lignocellulose
12. AFEX ----------Ammonia Fiber Expansion
13. XRD---------- X-Ray Diffraction
14. FTIR---------- Fourier Transform Infrared
15. ATR ----------Attenuated Total Reflectance
16. GC-MS------- Gas Chromatography-Mass Spectrometry
17. NIST---------- National Institute of Standard Technology
18. UV-VIS ------- Ultraviolet-Visible Light
19. RT ----------Retention Time
20. COD---------- Coefficient of Dispersion

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CHAPTER ONE

1.0 Introduction

1.1 Background

Concerns over declining fossil fuel reserves and resulting price increases, rising in energy needs resulting from burgeoning global population and strong industrialization drive, rising environmental issues and growing concerns about global security have sparked significant research and development projects aiming at creating transportation biofuels like ethanol and butanol all over the world. Because feedstock costs significantly affect the economics of producing biofuel, these programs place an emphasis on using commercially viable ($24–60/ton) industrial byproducts and agricultural waste, instead of molasses or maize (Qureshi & Maddox, 1992; Manderson, Spencer, Paterson, Qureshi, & Janssen, 1989; Qureshi & Manderson, 1995; Schoutens & Groot, 1985; Marlatt & Datta, 1986). "Ethanol is one of the actively pursued renewable biofuels due to its technological maturity and its favorable features as a transportation fuel, including its high-octane rating, low cetane number, and higher vapour pressure" (Wyman, 2018).

At the COP21 summit in 2015, the 193 United Nations members ratified a worldwide climate mitigation pact, pledging to cut greenhouse gas (GHG) emissions as part of the fight against climate change. With this accord, the average global temperature increase will be limited to 2 °C. (Hassan, Williams, & Jaiswal, 2019; United Nations, 2015).

The transportation sector is among the primary contributors of greenhouse gas emissions today. It is believed that one of the most important opportunities for assisting in the achievement of the COP21 targets is to decrease greenhouse gas emissions in this industry by switching to fuels with lower carbon intensity from fossil-based fuels (United States Environmental Protection Agency [USEPA], n.d.; Government of Canada, n. d). Modern combustion engines can use bioethanol in combination with gasoline with little to no

modification, allowing for a less disruptive shift away from fossil fuel source (Balat, 2011). Currently, 20 to 25 percent of anhydrous ethanol is added to gasoline in Brazil. Additionally, flex-fuel vehicles which may run on gasoline, alcohol, or both, have been available since 2003 (Mendonca et al., 2008). The main liquid biofuel utilized in transportation is ethanol. Ethanol made out of lignocellulosic biomass feedstock is a second-generation biofuel, G2. First-generation biofuel, G1, is produced from maize and sugar cane bagasse where the valorization sugar is glucose. "The low cost of second-generation biofuel substrates proposed by initiatives include agricultural residues (corn stover, wheat straw, barley straw, rice straw, sweet sorghum bagasse, and sugar cane bagasse), process coproducts (rice hulls, wheat bran, corn fiber, and glycerol), and energy crops (switchgrass, miscanthus, alfalfa, reed canary grass, and Napier grass)" (Saha, Nichols, Qureshi, & Cotta, 2011; Vertes, Qureshi, Yukawa, & Blaschek, 2011; Qureshi, 2010). According to recent estimates, the US could have 1.1 billion (1.1×10^9) tons of biomass accessible by 2030 for the manufacture of biofuels (**Qureshi, N., Singh, V., Liu, S., Ezeji, T. C., Saha, B. C., & Cotta, M. A. (2014)**; Lane, 2011). In order to lower costs and prevent a situation where food/feed and fuel are in conflict with each other and drive up the price of maize used by human and animal use, other feedstocks or substrates must be used for ethanol production to expand beyond current levels. "The successful bioconversion of this biomass into biofuels could replace approximately 30% of the 134.5 billion (134.5×10^9) gallons of transportation fuel used in the US per year" (Michael, 2014). Corn can be used to produce ethanol as well, and ethanol production reached 13.8 billion gallons in 2013, according to the Renewable Fuels Association's monthly U.S. fuels ethanol production/demand report, dated April 14, 2014 (Renewable Fuels Association [RFA], 2022). Once more, the US government has established a rule that by 2022, 36 billion gallons of renewable fuels must be produced annually. The development of innovative methods for effective and sustainable manufacture of biofuels from cellulosic biomass, a readily available source that does not threaten the food

supply, is necessary to achieve this goal (Fan, 2014). Producing biofuels from biomass can significantly contribute to achieving the country's transportation needs, reducing dependence on foreign oil, and tackling greenhouse gas emissions (Urbanchuk, 2011; Lynd, Weimer, Van Zyl, & Pretorius, 2002). Processing cellulosic biomass can also boost rural economies as well as increasing the demand for agricultural products, creating local green jobs, and advancing sustainability in our society (Lynd, Weimer, Van Zyl, & Pretorius, 2002; Lynd, Wyman, & Gerngross, 1999). Thus, achieving the aforementioned targets would require vigorous research with effective bioconversion of lignocellulosic biomass to bioethanol.

Plant materials which primarily consist of cellulose, hemicellulose, and lignin are called lignocellulosic biomass (Wiselogel, Tyson, & Johnson, 2018). Unrefined lignocellulosic biomass naturally comprises 40–55 % cellulose, 25–50 % hemicellulose, 10–40 % lignin, and around 5 % extractives and ash on a dry weight basis. According to the source of the biomass, different components are present in varying proportions (Wiselogel, Tyson, & Johnson, 2018; Sun, & Cheng, 2002).

Corn husk lignocellulosic biomass, the basis of this study, and based on chemical composition analysis has an approximate 31-39 wt % cellulose, 34-41 wt % hemicellulose, 2-14 wt % lignin, 3-7 wt % ash, and with an equilibrium moisture content of 9 wt % in accordance with the value recorded in literature (Mendes, Adnet, Leite, Furtado, & Sousa, 2015). This data shows favourable prospects for high sugar yield that would potentially result in high ethanol yield/production if the appropriate treatment conditions of the biomass are determined, and effectively bio-converting the lignocellulosic corn husk biomass to bioethanol. Knoema, (a privately held data technology business based in New York and launched in 2011), estimates that Ghana produced 3,000 thousand tonnes of maize in 2020. They stated that the production of maize in Ghana reached 3,000 thousand tonnes in 2020, up from 384 thousand tonnes in 1971, growing 8.79 % average yearly rate. These statistics show

that annually large volumes of corn husk are produced in the Ghanaian space, and with efficient economics in the process development, sustainability of an industrial implementation of a biorefinery project can be largely guaranteed.

1.2 Problem statement

Tonnes of agricultural wastes, industrial wastes, municipal solid wastes, woody wastes and forest biomass, wastes from green areas (parks, gardens), which are largely cellulose-based materials of which corn husk is inclusive, impose great challenge to Metropolitan Municipal and District Assemblies, (MMDAs) waste management systems across various jurisdictions including Ghana. Although research and development initiatives have made it possible for the utilization of these waste materials in various fields of study, nonetheless annual volumes of these materials overwhelm the MMDAs waste management systems. Unfortunately, most of these waste materials are discharged at land fill sites, left in the opening, and in the worst-case scenario, they are cheaply burnt at this land fill sites or directly on farm lands, therefore destroying the vegetation, the habitats of wildlife, producing greenhouse gases (GHGs) such as carbon dioxide (CO_2), the oxides of nitrogen (NO_x), resulting in global warming.

1.3 Significance of study

How would this study significantly add to scientific knowledge, especially at this time when most countries across the globe are targeting emission-free economics? Thus, taking a paradigm shift from the heavily dependent conventional non-renewable fossil fuels for the prospects of alternative and sustainable energy resources must be geared towards the right direction. Works done on the extraction of bioethanol is extensively on first generation bioethanol sources such as starch-and sucrose-based biomasses, utilizing the biological catalyst, *Saccharomyces cerevisiae*, as the lead to probe the extraction process. Thus, much is expected on the use of chemicals such as strong acids, nitrogen precursors, most especially on

lignocellulosic biomasses and/or bagasse of crops and plant by-products from which the juices or molasses were sourced to make first-generation bioethanol. Therefore, this study seeks to develop a chemical extraction model for high bioethanol yield from the assumed waste of lignocellulosic biomasses or bagasse, a G2 bioethanol source. The model uses a more subtle approach to achieve high sugar yield with a relatively much smaller volume of the feed acid as compared to other models that would produce relatively the same yield of sugar but with much larger volume of acid.

1.4 Aims/objectives of- study

Aims and objectives of the study include the following:

1 To extract bioethanol from corn husk lignocellulosic biomass.

2 To study the effects of sulfuric acid concentration and temperature on the yield of leached sugar prior to fermentation.

3 To study the effects of sulfuric acid concentration and temperature on the yield of ethanol after fermentation.

4 Characterization of the produced bioethanol; and evaluate its prospects for a potential biofuel.

1.5 Scope of work

The study on extraction of bioethanol from corn husk lignocellulosic biomass is reported in a five-chapter document. Chapter one focuses on the background of the study, and rationalizes the extraction of bioethanol from lignocellulosic biomass, projects a problem statement, outline the significance of the study, and lists key aims and objectives of the study. Chapter two takes a review of works done around the extraction of bioethanol from lignocellulosic biomass and bioethanol related works, the methodology: pre-treatment techniques, enzymatic hydrolysis, acid hydrolysis, microorganism fermentation, and product recovery utilized by researchers in the past. Chapter three reports the chemicals, materials and equipment;

systematically presents the chemical extraction model proposed by this study for the production of bioethanol from corn husk. Chapter four reports the results obtained in the preceding chapter, reports on characterization techniques and analytical results on the bioethanol produced, and discuss the results therein. Chapter five closes this study on the extraction of bioethanol from corn husk lignocellulosic biomass with conclusion remarks on the findings of the study and making recommendations for future perspectives.

CHAPTER TWO

2.0 Literature Review

2.1 First- and Second Generation- Biofuels

First generation biofuel, or G1, is produced from sources that are based on sugar or starch and accounts for close to 80% of the world's production of liquid biofuels. Fig. 2.1 depicts the distribution of fuel ethanol production around the world in 2012, corn served as the main raw material for approximately 70% of the fuel ethanol produced. The United States is by far the biggest fuel ethanol producer, producing 13.3 billion gallons in 2012 almost entirely from maize starch. Brazil is the second-largest producer, producing 6.0 billion gallons from sugar cane (both juice and molasses) in the same year (Barros, 2012).

Figure 2.1 Relative global production of fuel ethanol by region and by feedstock for 2012.

However, lignocellulosic biomass feedstock is used to make second-generation biofuel, G2. Lignocellulose is a possible raw material for biofuel production, including the biological synthesis of metabolites like ethanol from sugars obtained from plant cell wall polysaccharides. Many second-generation biofuel pilot plants have been in existence for a long time utilizing a variety of pretreatments and fermentation techniques, with numerous plants starting in 2014 (Brown, & Brown, 2013). “Agricultural residues (corn stover, wheat straw, barley straw, rice straw, sweet sorghum bagasse, and sugar cane bagasse), process

coproducts (rice hulls, wheat bran, corn fiber, and glycerol), and energy crops (switchgrass, miscanthus, alfalfa, reed canary grass, and Napier grass)" are among the low-cost substrates suggested by the programs (Saha, Nichols, Qureshi, & Cotta, 2011; Vertes, Qureshi, Yukawa, & Blaschek, 2011; Qureshi, 2010).

2.2 Relative Cost of Feedstock

Because feedstock prices have a substantial effect on the economics of producing biofuel, these programs place an emphasis on using commercially viable ($24–60/ton) agricultural leftovers and industrial byproducts instead of corn or molasses (Qureshi & Maddox, 1992; Manderson et al., 1989; Qureshi & Manderson, 1995; Schoutens, & Groot, 1985; Marlatt & Datta, 1986). Because maize is used to produce food, feed, and fuel, many companies are now in competition for it, which raises the price of the commodity. Fuel might be made from commercially viable, renewable agricultural wastes to avoid this competition. Compared to corn, which costs about $275 per ton, the cost of lignocellulosic wastes is $50 to $60 per ton (Qureshi, Saha, Cotta, & Singh, 2013; Lane, 2011).

2.3 Processing Routes

When sugars are used as reaction intermediates and enzymatic hydrolysis is used to produce ethanol from lignocellulosic biomass, there are five procedures involved: "pre-treatment, cellulase production, enzymatic hydrolysis, microbial fermentation, and product recovery". Consolidated bioprocessing, or CBP, is an economically feasible method of producing Biofuel figure2.3)

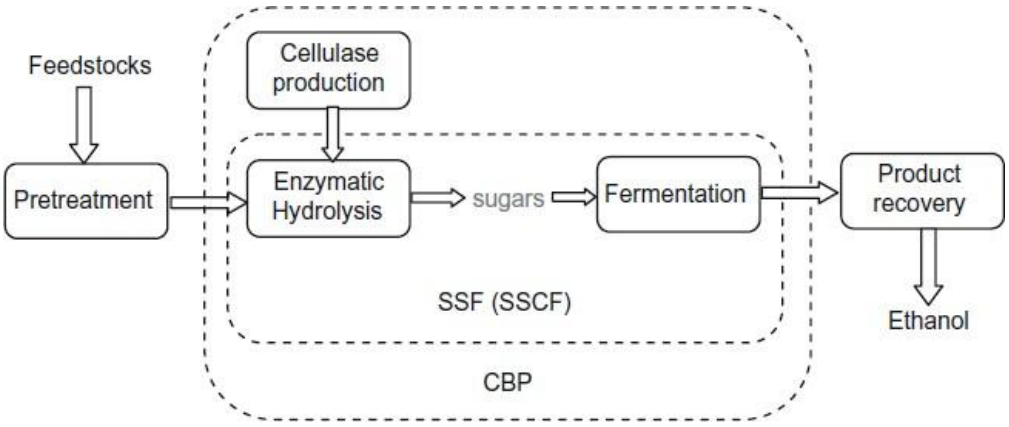

Figure 2.2 The process of ethanol production from lignocelluloses biomass

"If pre-treatment, cellulose hydrolysis, fermentation, and product recovery take place in different reactors the process is called separate hydrolysis and fermentation (SHF)". In this setup, the pre-treated material is mixed with cellulases from the enzyme manufacturing step to produce glucose from the cellulose fraction. Once the sugar has been hydrolysed, the fermentative microbe is added to produce ethanol. Enzymatic cellulose hydrolysis and hexose fermentation are combined in one reactor during simultaneous saccharification and fermentation (SSF) (Wright, 1988). Given that the fermenting microbe transforms glucose, a cellulase inhibitor, into ethanol, SSF can effectively remove or diminish the inhibitory impact of glucose on cellulases, resulting in quicker biomass hydrolysis rates and higher ethanol yields in comparison to SHF. Due to sophisticated engineering approaches that allow the fermentative microbe to impart both five- and six carbon- sugars, the simultaneous saccharification and fermentation (SSCF) process consolidates enzymatic hydrolysis, hexose and pentose fermentation, and other processes into one phase (Ho, Chen, & Brainard, 1998; Zhang, Eddy, Deanda, Finkelstein, & Picataggio, 1995; Ingram et al., 1999). SSCF can produce higher product yields and more efficient processes than SSF. Finally, consolidated

bioprocessing (CBP) combines cellulase synthesis, enzymatic cellulose hydrolysis and fermentation in a single step.

Alternatively, the use of enzymatic cellulose hydrolysis in the production of the sugars and further fermentation using a microorganism is acid cellulose hydrolysis. The process involves pretreating the biomass, performing acid hydrolysis, fermenting the hydrolysate and recovering ethanol from the fermentation broth.

Researchers use several variations of the concentrated acid-hydrolysis method, but there are typically two phases involved. Wolfaardt *et al.*, (2021) illustrate a schematic diagram of the concentrated acid hydrolysis and fermentation process as shown in Figure 2.3. The first step, also known as swelling, fractionation, or de-crystallization, aims to break the lignin sheath (and the covalent bonds between lignin and cellulose and hemicelluloses), solubilize hemicelluloses, and decrease the crystallinity and degree of polymerization of cellulose in order to break the biomass' recalcitrance (Baruah, Das, & Sharma, 2018). This process is normally carried out at high solid-liquid ratios (1:2.5 to 1:10(w/v)), low temperatures (60 oC), and high acid concentrations (>25 wt %) (Oriez, Peydecastaing, & Pontalier, 2020). The residual hemicelluloses, polymers, and oligomers are hydrolyzed to their monomeric forms in the second step, also known as post-hydrolysis. Higher temperatures (about 70-120 ^{o}C or above) and lower acid concentrations are often used for this stage (Balat, 2011). This phase normally uses one of two acid concentration ranges: the more conventional concentrated acid hydrolysis technique with an acid concentration range of 20-30 wt % acid (Janga, Hägg, & Moe, 2012; Farone & Cuzens, 1998; Liu, Wu, Kida, & Tang, 2012; Heinonen & Sainio, 2010), or a more dilute concentration range of 0.5-10 wt % (Sun, Tang, Iwanaga, Sho, & Kida, 2011; Chen, 2011; Helland & Weydahl, 2002; Hoshino et al., 2007). There are various acid recovery points that have been used in literature (as indicated by points 1, 2 and 3 i

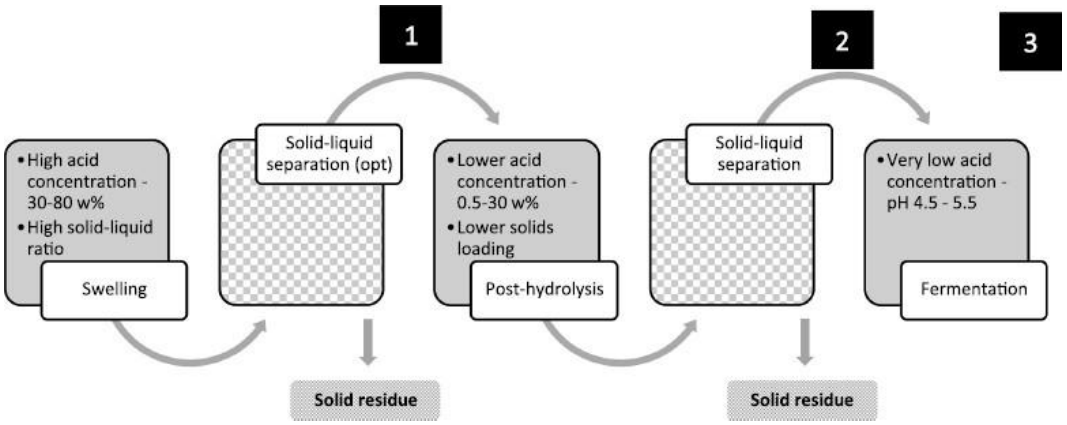

Figure 2.3 A high-level schematic diagram of concentrated acid hydrolysis and fermentation process with possible points of acid recovery.

2.4 Sample Pretreatment Techniques

Strong intramolecular and extra-molecular hydrogen bonds characterize cellulose, which is extremely crystalline and intertwined with hemicellulose and bordered by lignin (Hatfield, Ralph, & Grabber, 1999). As a result, direct enzymatic hydrolysis of lignocellulosic biomass is challenging. Therefore, pre-treatment is required to raise the accessibility of cellulose for enzymatic hydrolysis. This is accomplished by breaking the lignin seal, solubilizing the hemicellulose, and reducing the degree of cellulose crystallization. To accomplish the aforementioned objectives, a variety of physical, thermal, and biological approaches have been used over time (Hsu, 2018; Himmel, Baker, & Overend, 1994). The highest yields and lowest costs for boosting the release of sugars from cellulosic biomass in subsequent hydrolysis have, to date, come from chemical pre-treatment of cellulosic biomass at elevated temperatures. The most widely utilized chemicals are lime, sulphuric acid, ammonia, and sulphur dioxide (Mosier et al., 2005). An efficient pre-treatment must restore high sugar concentration while constantly preventing their loss and degradation; it must also diminish inhibitor development, allow fermentation to proceed without detoxification, and consume

less water and energy to save expenses (Galbe & Zacchi, 2012; Sarkar, Ghosh, Bannerjee, & Aikat, 2012; Sun & Cheng, 2002).

2.4.1 Acid Pretreatment

Fatmawati and Agustriyanto (2015) and da Costa Nogueira *et al.*, (2018) report that to get large yields of sugars from lignocellulosic biomass, a common and efficient method is acid pre-treatment. Inasmuch as this statement, to a large extent maybe true, however, the structure of the biomass differs depending on the substrate., thus factors such as feed acid concentration, temperature must be critically tested to suit a particular biomass.

2.4.2 Alkaline Pretreatment

Alkaline pre-treatment has two main effects: delignification of the biomass and a decrease in crystallinity (Galbe & Zacchi, 2012; Sarkar, Ghosh, Bannerjee, & Aikat, 2012; Sun & Cheng, 2002; da Costa Nogueira et al., 2018). For these pre-treatments, "sodium, potassium, calcium, and ammonium hydroxide and ammonia are used" (Sarkar, Ghosh, Bannerjee, & Aikat, 2012; Sun & Cheng, 2002). In their work, in order to produce greater sugar concentrations, Soares et al. (2016) suggested pre-treating the biomass with diluted NaOH (1 % (w/v)) at room temperature to reduce the production of inhibitors. They also suggested using solid loadings (18% (w/v)) instead of detoxifying the pre-treated biomass.

2.4.3 Steam Explosion Pretreatment

In their review work on bioethanol production from lignocellulosic biomass, Saraswat and Chokshi (n.d) report that high-pressure, high-temperature steam is introduced into a closed chamber containing chips of lignocellulosic material. When pressure is removed after 1 to 5 minutes, steam expands inside the lignocellulosic matrix, separating the individual fibers and igniting a decomposition explosion. Before the material is exposed to atmospheric pressure for cooling, a temperature of 433-533 K and a pressure of 0.69-4.83 MPa are maintained. A

reduction in time and temperature, improved hydrolysis, a decrease in the production of inhibitory chemicals, and the complete elimination of hemicellulose can all be achieved by adding acids like H_2SO_4 (or SO_2) or CO_2 during a steam explosion. Economically, steam explosion pre-treatment is appealing since it recovers 45–65 % of xylose. It has been demonstrated that this procedure encourages delignification. The chemicals utilized are less dangerous. Less damage to the environment and completely recovering sugar (Hahn-Hägerdal, Galbe, Gorwa-Grauslund, Lidén, & Zacchi, (2006). "But it is high energy intensive and less efficient than SPORL and Organsolv pretreatment" (Demirbas, & Balat, 2006).

2.4.4 Organosolv Pretreatment

Burning the liquor is not necessary when using an organic solvent and water mixture since the lignin can be separated without burning (by distillation of the organic solvent). It takes 30 to 60 minutes with 40 to 60 percent of an organic solvent at 160 to 190 oC (Joshi et al., 2011). Saraswat and Chokshi (n.d) cited the use of 90 % formic acid and pressurised CO_2 in combination, recommending the use of other solvents such as methanol, ethanol, acetic acid, peracetic acid, acetone, etc.

2.4.5 Sulphite Pretreatment to Overcome Recalcitrance of Lignocellulose (SPORL)

To weaken the chemical structure, SPORL uses aqueous sulphite or bisulphite solution over a wide pH and temperature range. Reagent can be used to change the pH of pre-treatment liquid. Therefore, the energy needed for mechanical processes is reduced by more than ten times. It is very scalable for commercial manufacturing (Gerlach, 2012).

2.4.6 Ammonia Fiber Expansion (AFEX)

A pressure vessel is used to hold prewetted lignocellulosic material that has a moisture content of 15–30 %. This material is then exposed to liquid ammonia at high temperatures and pressures, followed by a quick breakdown. Depending on how much the biomass is

saturated, the residence duration can range from short (5–10 min) to intermediate (30 min) at a temperature of 60–100 ^{o}C. The ammonia loading is about 1-2 kilogram of ammonia per kg of the dry substrates. Pressures greater than 12 atm are necessary. There are no inhibitors created, and very fine feed is not necessary (Saraswat and Chokshi (n.d)). It is easy and takes little time. This technique is ineffective with biomass with high lignin content, (e.g., softwood newspaper) (Sarkar, Ghosh, Bannerjee, & Aikat, 2012).

2.5 Hydrolysis

One of the main objectives of research and development on renewable resources is the cost-effective hydrolysis of cellulose to make fermentable sugars for the production of chemicals and fuels. Enzymes or an acid are commonly used to achieve cellulose hydrolysis. As a result of steric issues, enzymatic hydrolysis of lignocellulosic materials progresses slowly. Acid hydrolysis of lignocellulosic materials results in a decrease in yield (Lipinsky, 1979).

2.5.1 Acid Cellulose Hydrolysis

Saraswat and Chokshi (n. d) report that Two steps make up the process of dilute acid hydrolysis. The first stage uses 0.7% H_2SO_4 and 462 K at low temperature to boost hemicellulose yield, and the second stage uses 0.4% H_2SO_4 and 488 K at higher temperature to maximize cellulose hydrolysis. Continuous processing is facilitated by the quick rate of reaction. While sustaining a rapid rate of cellulose hydrolysis and minimal glucose breakdown, it can be difficult to increase glucose output by more than 70% economically. The feedstock size for quick continuous processes must also be decreased to mm in order to allow appropriate acid penetration. Concentrated acid hydrolysis utilizes 70 % H_2SO_4 at 313-323 K for 2-4 hrs in the reactor. Low pressure and temperature prevent significant sugar deterioration. For additional cellulose hydrolysis, the solid residue from the first step is dewatered and submerged in 30 to 40% concentrations of sulfuric acid for 50 minutes at 373 K. This results in low degradation and up to 100% yield when compared to dilute acid

hydrolysis. Although the production of inhibitors is less, it is economically less appealing since it takes significant amounts of acid and expensive acid recycling.

2.5.2 Enzymatic Cellulose Hydrolysis

Cellulases are used in the process of enzymatic hydrolysis to breakdown pretreated lignocellulosic biomass into fermentable sugars. The method entails the following crucial steps: "(1) the transfer of enzymes to the surface of the cellulose from the bulk aqueous phase, (2) the adsorption of the enzymes and the formation of enzyme-substrate complexes, (3) the hydrolysis of the cellulose, (4) the transfer of the hydrolysis products from the surface of the cellulosic particles to the bulk aqueous phase, and (5) the hydrolysis of cellodextrins and cellobiose to glucose in the aqueous phase" (Walker & Wilson, 1991; Lee, Shin, Ryu, & Mandels, 1982; Ladisch, Gong, & Tsao, 1980; Woodward, Hayes, & Lee, 1988). The structural characteristics of lignocellulosic biomass, as well as the content and source of the cellulases, have an impact on the overall rate of the process. (Lynd, Weimer, Van Zyl, & Pretorius, 2002; Walker & Wilson, 1991; Yang, Dai, Ding, & Wyman, 2011).

"Achieving complete hydrolysis of cellulose into monomer sugars requires the synergetic effects of three major types of enzymes: endo-β-1,4-glucanases (EC3.2.1.4), cellobiohydrolases (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21)" (Teeri, 1997). Endoglucanases (EGs) break the more amorphous cellulose chains in half, releasing more free ends as a result. Cellobiohydrolases (CBHs) release soluble cellooligosaccharide with cellobiose as the main product after depolymerizing and hydrolyzing the highly crystalline portions of cellulose from the ends of the chain. Finally, β-glucosidases (BGLs) hydrolyze cellooligosaccharides into monomer glucose (Teeri, 1997). The modular structure of cellulases is another crucial characteristic in general. Catalytic and carbohydrate-binding modules are frequently seen in cellulases (CBMs). The catalytic domain is brought close to the substrate by the CBMs' binding to the cellulose surface. The processivity and initiation of

exoglucanases are particularly dependent on the kind of CBMs (Teeri, Koivula, Linder, Wohlfahrt, Divne, & Jones, 1998).

2.6 Microorganism Fermentation

The process of producing ethanol from sugars dates back thousands of years, and the technology for producing ethanol fuel from feedstocks based on starches or sugars is well established. The employment of the microbial strain Saccharomyces in this method of fuel generation is essential for the quick and effective manufacture of ethanol from the sugars. However, the prospective microorganism must meet other requirements in order to produce ethanol from cellulosic biomass. Contrary to corn hydrolyzate, which solely releases glucose as a monomer, lignocellulosic biomass hydrolyzate also contains pentoses and hexoses. Therefore, cofermenting the various sugars requires a microbial strain that has undergone genetic modification.

In the simplest terms, enzymes function sequentially to convert 1 mole of glucose into 2 moles of ethanol and 2 moles of carbon dioxide during the fermentation of carbohydrates into ethanol by yeast:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 \quad \text{.....(1)}$$

The greatest predicted yield for producing ethanol from glucose (YEtOH/Glc) according to these metabolic pathways is 2.0 molmol-1, or 0.511 gg-1. However, because some carbon is used to form glycerol, other minor metabolites, and cell biomass, industry normally generates yields between 90 and 95 percent of the theoretical maximum (Häggström, Rova, Brandberg, & Hodge, 2014). To obtain favourable economics, it is critical that each sugar in the hydrolyzate is transformed by fermenting bacteria. The fermenting microorganisms must be able to tolerate these inhibitors and display great process robustness because several inhibitors are created throughout the pretreatment process (Zaldivar, Nielsen, & Olsson, 2001). Two methods are utilized to create microbes that are able to make ethanol at high

concentrations and yields from all of the sugars found in cellulosic biomass hydrolyzate (Zaldivar, Nielsen, & Olsson, 2001; Lynd, Weimer, Van Zyl, & Pretorius, 2002; Lynd, Wyman, & Gerngross, 1999). The native cellulolytic approach entails locating a microbe with a broad range of substrates and then transforming it into a productive ethanologen. Starting with a top-notch native ethanologen and expanding its spectrum of substrates are key components of the recombinant cellulolytic method. Metabolic engineering must be used to implement both tactics (Zaldivar, Nielsen, & Olsson, 2001; Lynd, Weimer, Van Zyl, & Pretorius, 2002; Lynd, Wyman, & Gerngross, 1999).

2.6.1 *Saccharomyces cerevisiae and Zymomonas mobilis*

For building ethanologens for biomass conversion using the recombinant technique, *Saccharomyces cerevisiae* and *Zymomonas mobilis* are appealing starter microorganisms. They have been extensively utilized in industry to produce ethanol from feedstocks with a starch- or sugar-based composition (Zaldivar, Nielsen, & Olsson, 2001; Lynd, Weimer, Van Zyl, & Pretorius, 2002). They have numerous advantageous qualities, including as high productivity and ethanol yield, high ethanol tolerance, process hardiness, and generally recognized as safe (GRAS) status (Zaldivar, Nielsen, & Olsson, 2001; Lynd, Weimer, Van Zyl, & Pretorius, 2002). However, neither of these organisms can utilise cellobiose, xylobiose, or pentose sugars; they can only use hexose monomers in the cellulosic biomass hydrolyzate (Zaldivar, Nielsen, & Olsson, 2001; Lynd, Weimer, Van Zyl, & Pretorius, 2002). The substrate range of *S. cerevisiae* has been extended, and efforts have been undertaken to allow *S. cerevisiae* to ferment xylose and arabionose (Ho, Chen, & Brainard, 1998; Hahn-Hägerdal, Karhumaa, Fonseca, Spencer-Martins, & Gorwa-Grauslund, 2007). Piromyces sp. in *S. cerevisiae* produced a functioning xylose isomerase recently, which solved the redox imbalance issue brought on by the earlier attempt employing a xylose reductase (XYL1) and xylitol dehydrogenase (XYL2) system (Kuyper et al., 2003). The recombinant strain grew on

cellobiose at almost the same pace as on glucose under anaerobic circumstances when *S. cerevisiae* was engineered to produce a recombinantly secreted BGL from Saccharomycopsis fibuligera (Van Rooyen, Hahn-Hägerdal, La Grange, & Van Zyl, 2005). Recently, cellulodextrin transporter and intracellular BGL from Neurospora crassa were expressed in S. cerevisiae to create cellobiose-utilizing yeast. The recombinant strain effectively transports cellobiose and intracellularly hydrolyzes it to produce glucose (Galazka et al., 2010). Cellobiose and xylose can both be consumed concurrently by a recombinant strain that also expresses the xylose consumption pathway (Ha et al., 2011). Progress has also been achieved in extending the substrate range of *Z. mobilis*, including enabling *Z. mobilis* to ferment xylose (Zhang, Eddy, Deanda, Finkelstein, & Picataggio, 1995), coferment xylose and arabionose (Deanda, Zhang, Eddy, & Picataggio, 1996; Mohagheghi, Evans, Chou, & Zhang, 2002; Mohagheghi, Evans, Finkelstein, & Zhang, 1998), and tolerate higher concentrations of acetate and other inhibitors in the cellulosic biomass hydrolysate (Jeon, Svenson, Joachimsthal, & Rogers, 2002; Joachimsthal, Haggett, Jang, & Rogers, 1998; Ranatunga, Jervis, Helm, McMillan, & Hatzis, 1997).

2.6.2 *Escherichia coli and Klebsiella oxytoca*

Enteric bacteria like *Escherichia coli* and *Klebsiella oxytoca* are very desirable beginning microorganisms in the local approach for developing microorganisms that ferment sugars in cellulosic biomass because they have a broad range of substrates and utilize both hexose and pentose sugars in cellulosic biomass hydrolyzate (Lynd, Wyman, & Gerngross, 1999; Zaldivar, Nielsen, & Olsson, 2001; Ingram *et al*., 1999). *K. oxytoca* can transport and use all of the monomer sugars found in the lignocellulosic biomass, as well as their oligomers, including cellobiose, xylobiose, and xylostriose (Wood & Ingram, 1992). By expressing the Z. mobilis homoethanol route, it was possible to transform wild-type E. coli into a productive ethanologen (pyruvate decarboxylase and alcohol dehydrogenase). The recombinant strain in

a rich medium was able to produce ethanol at a rate that exceeded 90% of the predicted yield from glucose (Ingram, Conway, Clark, Sewell, & Preston, 1987). Later effort has been done to increase the output of ethanol (Ohta, Beall, Mejia, Shanmugam, & Ingram, 1991), to enable better growth in a lean medium (Yomano, York, Zhou, Shanmugam, & Ingram, 2008), and to coferment glucose and xylose, which accelerated the metabolism of a five-sugar mixture (mannose, glucose, arabinose, xylose, and galactose) to ethanol (Yomano, York, Shanmugam, & Ingram, 2009). *K. oxytoca* P2 was created after the successful introduction of the *Z. mobilis* homoethanol pathway, which can convert a variety of carbohydrates into ethanol at a rate greater than 90% of the theoretical yield (Wood & Ingram, 1992). Work has since been done to increase the output of ethanol from carbohydrates (Wood, Yomano, York, & Ingram, 2005). However, compared to native ethanologens like *Z. mobilis* or *S. cerevisiae*, both *E. coli* and *K. oxytoca* are less tolerant to ethanol (Ingram *et al.*, 1999).

2.7 Product Recovery

The primary technique for recovering ethanol from fermentation broth is distillation. In a so-called beer column, ethanol is often produced using a distillation or stripping process at a concentration of 90% or more. After distillation, anhydrous ethanol is created using further procedures such gas-phase molecular sieve adsorption, additional distillation using a recovered entrainer, pervaporation, and other membrane-based activities (Lynd, Elander, & Wyman, 1996; Lynd, 1996).

Due to biological and processing limitations, the concentration of ethanol in the fermentation broth made from cellulosic biomass is substantially lower compared to that which is made from corn-based feedstocks (Wu & Lee, 1997). In a normal concurrent saccharification and fermentation (SSF) procedure, a solid content value of more than 15% in the broth is challenging to manage (Wu & Lee, 1997). The ethanol percentage in the broth (with a 15% solid content) is roughly 5% at carbohydrate concentrations typical for cellulosic feedstocks.

Further, cellulase and the fermenting microbe are both inhibited by ethanol at concentrations greater than 60 g/L (Holtzapple, Cognata, Shu, & Hendrickson, 1990; Ghose & Tyagi, 1979). When ethanol levels in the fermentation broth drop, product recovery costs rise, and to ensure profitable ethanol recovery, ethanol concentrations must be higher than 40 g/L (Zacchi, & Axelsson, 1989).

CHAPTER THREE

3.0 Experimental and Methodology

3.1 Chemicals, Materials and Equipment

The corn husk biomass used for this work was obtained from a local farming community in the Greater Accra Region, Ghana. The fermenting microbial strain, *Saccharomyces cerevisiae* was obtained from Allinson Dried Active Yeast. The chemicals used were sulfuric acid (H_2SO_4 at 98% purity), potassium metabisulfite ($K_2S_2O_5$ at 98%), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$ at 97%), ethanol (CH_3CH_2OH at 99.8%) were all obtained from Sigma Aldrich. Also, sucrose in the form of ordinary granulated sugar was obtained from United Sugars Corporation. The equipment used are an Abbe 60 refractometer from Bellingham-Stanley, Hammer milling machine from Christy Turner Ltd, Alpha FTIR spectrophotometer from Brukker, Clifton water bath, rotary evaporator, Stuart magnetic stirrer, and a Hanna (HI 2211pH/ORP) pH meter.

3.2 Sample Preparation and Acid Hydrolysis

The corn hush biomass was washed with deionized water to disengage the sample from other unwanted materials such as grains of particles of sand, other plant materials, and so on. It was sun-dried for 48 hrs, and shredded to much smaller size particles. The shredded mass was then milled and sieved using the hammer milling machine, obtaining an average particle size of 100 µm. This treatment is essential such that obtaining fine particle size raises the surface area, therefore increasing the rate of hydrolysis.

Sulphuric acid solutions of concentrations, 0.5 wt%, 10 wt%, 20 wt% and 30 wt% were made from a stock of 98 wt% purity. 4 g of the milled corn husk was poured into each of four 250 ml glass beakers. 40 ml of each acid solution was added to the milled corn husk in each beaker. The mixtures were each homogenized on a magnetic stirrer at 300 rpm for 20 mins.

The mixtures were heated on a water bath at 50 ^{o}C for 1 hr and allowed to cool at room temperature. After a 24-hr period, 2 ml, 40 ml, 80 ml, and 120 ml of deionised water were added to the corn husk mixtures of concentrations, 0.5 wt%, 10 wt%, 20 wt%, and 30 wt%, respectively, thereby reducing each feed acid concentration to 10 wt%. The mixtures were again homogenized on a magnetic stirrer at 300 rpm for 20 mins and heated on a water bath at 60 ^{o}C for 1 hr. The mixtures were allowed to cool at room temperature and left for 24 hrs. They were then filtered to obtain an aqueous hydrolysate. Brix tests were done on each aqueous hydrolysate and the readings were recorded. The process was repeated at 80 ^{o}C and 100 ^{o}C. All three different hydrolysis temperatures and their Brix values are recorded on Table 4.1. The highest Brix value, 33^{o} was produced from the feed acid concentration of 30 wt% and at 80 ^{o}C and the hydrolysate was used for fermentation.

3.3 Brix and pH Adjustment

A refractometer was used to measure the Brix of the hydrolysate. The hydrolysate in a beaker was placed in the refractometer's prism and the lid closed. The control knob was adjusted perpendicularly to a light source and viewed through to see the internal scale. The Brix reading is where the light and the dark areas meet on the scale. Thus, with the aid of the objective lens the Brix reading was taken. The Brix value was 33^{o}, and the pH 1.8. A solution of 1M NaOH solution was added to the hydrolysate, increasing the pH to 6.5. The Brix of the hydrolysate after the pH adjustment was measured to be 13^{o}. The 13^{o} Brix value was ameliorated with 70 % sucrose solution (prepared from ordinary white granulated sugar) and raised the Brix content to 20^{o}, which is the recommended Brix value for effective microorganism fermentation.

The 20^{o} Brix hydrolysate was sterilized using 150 ppm potassium metabisulphite and 0.3 g/L diammonium hydrogen phosphate, which is to provide a nitrogen source for the fermentation process.

3.4 Fermentation of Hydrolysate and Ethanol Recovery

A genetically modified variant of the microbial strain *Saccharomyces cerevisiae* was used in the fermentation process. For a hydrolysate volume of 280 ml, 4.2 g of the microorganism was weighed. The dry yeast was then activated with a portion of the hydrolysate by addition of yeast (2 g/100 ml) to a volume of the hydrolysate and incubated for 45 mins at 25 ^{o}C. After the incubation, the activated yeast solution was then added to the main solution. The vessel containing the fermenting solution was allowed to be partially aerated and was stirred on daily basis to liberate the yeast in the solution. The fermentation lasted for the first 14 days to form a broth.

The ethanol in the fermentation broth was separated by distillation using a rotary evaporator, commonly referred as reduced pressure evaporation. The fermentation broth was put into a round bottom flask that could rotate freely and partially submerged in a water bath set at 78 ^{o}C. A suction pump was used to evacuate the air molecules in the device compartment, reducing the pressure, and therefore enhancing the flow of the vapour to reach the condenser which was packed with crumbs ice. The condensate drips into a receiving flask. The process lasted for a period of two and half hours. Eventually, the rate of the distillate dripping into the receiving flask was almost zero, showing the completion of the distillation at this first stage. The process was repeated with the recovered distillate which lasted at a relatively shorter time.

Figure 3.1 is a process flow of the extraction of bioethanol from corn husk. It details the entire extraction process, illustrating quantities of materials used, treatment conditions and some measured results.

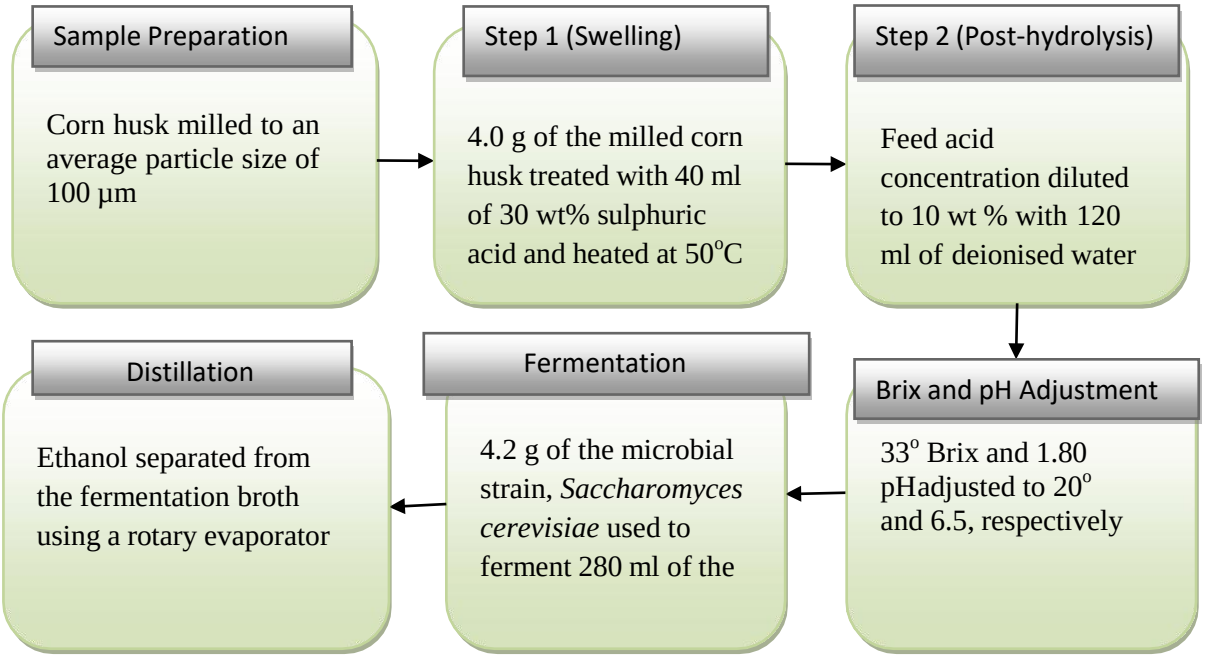

Figure 3.1 Process flow of the extraction of bioethanol from corn husk.

3.5 Characterization Techniques and Methods

X-Ray powder diffraction (XRD) spectroscopy patterns were obtained for the corn husk and the residual solid residue generated during hydrolysis as shown in Fig.4.2. The powders were further ground into fine powder and loaded into a sample holder which was transferred into a spinner stage in the X-ray diffractometer (Pananalytical Empyrean series) with Cu tube using Kα line at 1.54 Å. The Kβ line was removed with a nickel filter. Data was collected from start angle zero 2θ degrees to end angle hundred 2θ degrees. The tube was at 45 kV, 20 mA.

Fourier Transform Infrared (FTIR) spectroscopy was conducted on the distillate to identify the functional groups present. The analysis was done using FTIR spectrometer from Bruker connected to a computer with OPUS operator manager software. The instrument was operated under standard conditions and set to blank. 1µl of the sample was dropped on the attenuated total reflectance (ATR) without being in contact with the probe. An operation

command was issued and the spectrum was displayed on the computer screen as shown in Fig.4.3.

Gas chromatography-mass spectrometry (GC-MS) analysis was done to qualitatively identify the chemical impurities present in the distillate. A PerkinElmer GC Clarus 580 Gas Chromatograph with a PerkinElmer Clarus SQ 8 S Mass Spectrometer equipped with a ZB-5HTMS (5% diphenyl/95% dimethyl polysiloxane) fused to a capillary column (30 x 0.25 m ID x 0.25 m DF) was utilized to conduct the GC-MS analysis of the material. The oven's temperature was set to start at 80 °C and rise 15 °C per minute to 150 °C, then 3 °C per minute to 250 °C, and remain for 6 minutes at 250 °C. An ionization device with ionization energy of 70 eV was used for the GC-MS detection in electron impact mode. With an injection volume of 1µl and a constant flow rate of 1.6 ml/min, helium gas (99.999%) was used as the carrier gas. The ion source temperature was 220 ^{o}C, while the injection temperature was kept at 250 ^{o}C. With a scan interval of 0.5 seconds and a range of 45 to 450 Da, mass spectra were obtained at 70 eV. The GC/MS running time was 44 mins overall, and the solvent delay ranged from 0 to 3 mins. This analysis used a Turbo-Mass mass-detector, and Turbo-Mass ver. 6.1.0 software was used to manage mass spectra and chromatograms. Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. Fig.4.4 is a chromatogram plot of the GC-MS analysis.

UV-vis spectroscopy analyses were done to ascertain the presence of chemical impurities in the distillate by comparing the spectrum of standard ethanol at 99.8 wt% to the spectrum of the distillate as shown in Fig. 4.5(a), and draw a calibration curve to determine the ethanol concentration. These analyses were performed using Genesys 10S spectrophotometer; model number G10S UV-Vis with a wavelength range of 190 nm - 1,100 nm. Standard ethanol at 99.8 wt% purity was used in preparing ethanol concentrations (5, 10, 15, 20, 25, 30, 40, 50,

and 100) v/v %. Deionised water was used as blank in setting the baseline. The instrument was scanned for the entire wavelength range for all nine prepared concentrations. The maximum wavelength for the ethanol absorption was 220 nm. The absorbance of each concentration was measured and that of the distillate. Fig. 4.5(b) shows the calibration curve of absorbance versus ethanol concentration. The absorbance of the distillate is extrapolated on the curve and the concentration of the distillate determined.

CHAPTER FOUR

4.0 Results and Discussion

4.1 Hydrolysis

Optimizing the relevant conditions, temperature and acid concentration to maximize sugar yield produced the following results as shown in Table 4.1.

Table 4.1 Hydrolysis conducted at 60 ^{o}C, 80 ^{o}C, and 100 ^{o}C using 4 g of corn husk.

H_2SO_4 Conc. (wt %)	**Temperature/60 ^{o}C**	**Temperature/80^{o}C**	**Temperature/100 ^{o}C**
	^{o}Brix	^{o}Brix	^{o}Brix
0.5	0.5	2.0	2.0
10	11.0	22.0	11.0
20	19.0	21.0	21.0
30	29.0	33.0	29.0

Table 4.1 shows the relationship between feed acid concentrations and the amount of sugar leached from the corn husk at three different temperatures. In all three different temperatures, (i.e., 60, 80, and 100) ^{o}C, increase in acid concentration varies linearly, at least for the (0.5 - 30) wt % range of acid used, with increase in the amount of sugar leached from the corn husk. The data, however, shows the best yield of sugar from a feed acid concentration of 30 wt% at 80 ^{o}C. Thus, the data indicates that the post-hydrolysis temperature of 80 ^{o}C optimizes the yield of sugar at 30 wt% acid concentration in the hydrolysis of corn husk. Fig.4.1 shows a plot of °Brix versus acid concentration v/v % for all three different post-hydrolysis temperatures from Table 4.1. The black, red, and blue graphs represent the post-hydrolysis temperatures 60°C, 80°C, and 100°C, respectively. The red graph shows a significant difference in the slopes of all three graphs. However, there is an outlier at the coordinate (20 wt %, 21^{o}). The Brix value of 21^{o} shows a deviation from the increasing trend as observed in the acid concentration range, (0.5-30 wt %). This may be attributed to some random

uncertainty rather than a systematic error since all the readings were taken by the same instrument and on the same day.

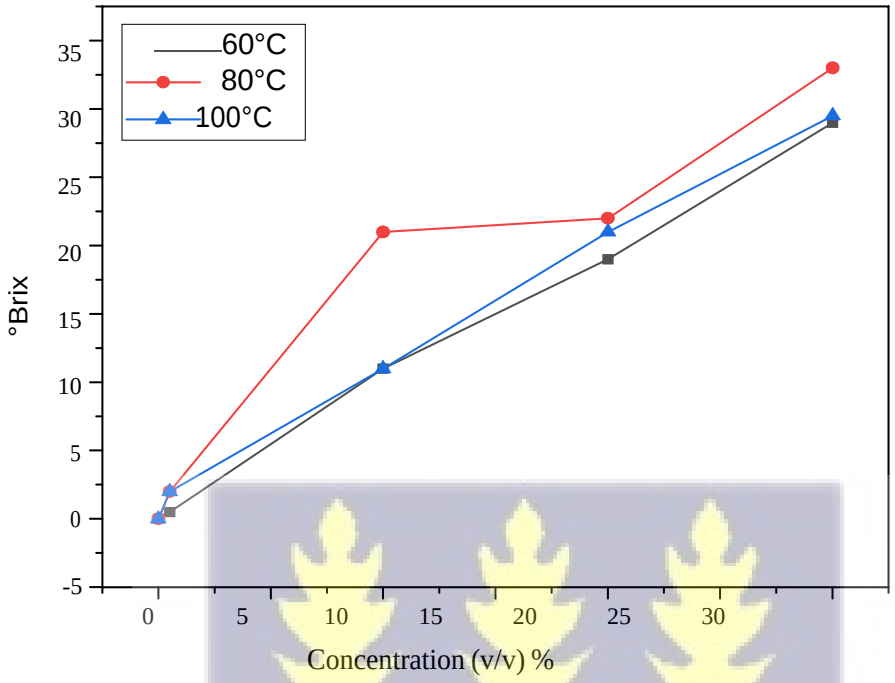

Figure 4.1 Plot of °Brix versus acid concentration v/v % at three post-hydrolysis temperatures.

4.2 Microorganism Fermentation

Results on fermentation are reported on gauging the activity of the yeast by measuring the Brix of the fermenting content on two occasions and estimating the yield of ethanol in the fermentation broth prior to distillation. The Brix of the solution was measured on the seventh day of the start of fermentation to gauge the activity of the fermenting microorganism, *Saccharomyces cerevisiae*. From the initial Brix value of 20°, the new Brix value was 16.5°, representing 17.5 % of the sugars being converted to ethanol. The second and last reading was taken on the fourteenth day (i.e., before distillation), and was found to be 14.8°, representing 26 % of the total sugars being converted to ethanol for the entire fourteen days period. The amount of ethanol in the fermentation broth is estimated at the end of

fermentation, using the initial Brix value of 20^{o} (i.e., before fermentation), and the final Brix value of 14.8^{o} (i.e., before distillation). An online computational tool imputed with the initial and final Brix values generated gravity values of 1.0830 and 1.0630, respectively, and gives an estimate of the ethanol content in the broth as 2.9 %. This value falls short of the recommended value of 4.0 % or more of ethanol from a lignocellulosic biomass source that has some economic viability. The 2.9 % ethanol content in the broth may be attributed to the fact that this variant of the yeast potentially coferment both pentose and hexose sugars but easily get denatured as the concentration of ethanol in the vessel increases with time.

4.3 Analysis and Characterization

The raw powdered corn husk, the residual solid residue generated during hydrolysis, and the distillate were subjected to a number of characterisation analysis.

4.3.1 X-Ray Powder Diffraction (XRD) Spectroscopy

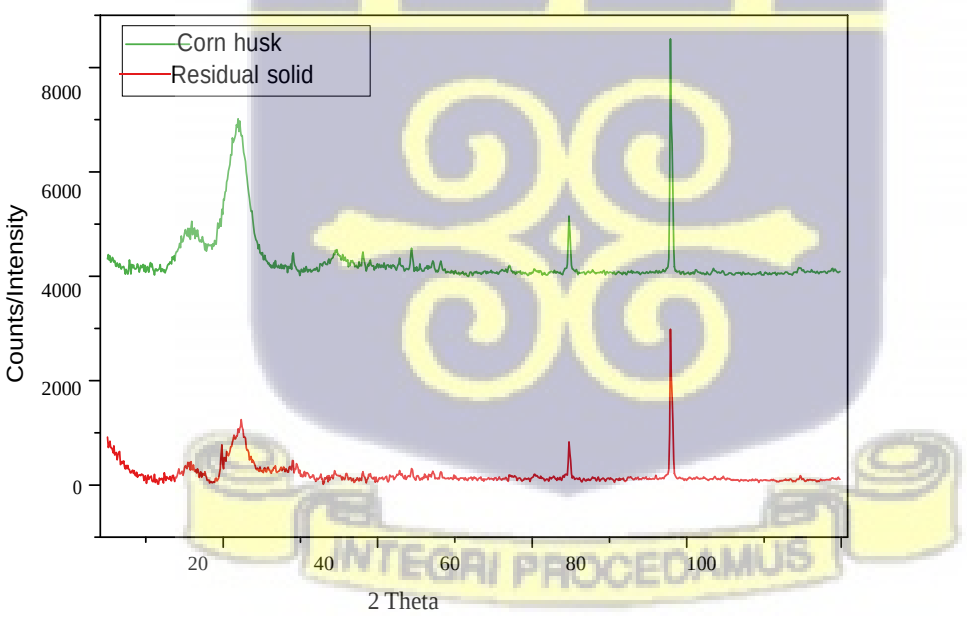

Figure 4.2 X-ray powder diffraction patterns of the corn husk and residual solid residue generated during hydrolysis.

The ordinate represents counts/intensity and the abscissa is 2θ. Four crystallographic peaks are clearly shown. From left to right are the 2θ values which show no significant difference for both graphs: 15.97°, 21.96°, 64.80°, and 77.92°. The respective counts for the corn husk are, 4121.73, 5726.32, 1657.39, and 4982.41. The intensities for the residual solid residue are, 2432.75, 3214.32, 1217.40, and 3289.39, respectively. These peaks are an indication of the crystalline nature of the cellulosic component of the corn husk since the lignin mass and the hemicellulose are mainly amorphous. There is significant difference in the respective counts between the two graphs. This difference in counts provides evidence of the breakdown of the crystalline cellulose of the corn husk. The percentage reductions in the four peaks are 49.78%, 43.87%, 26.55% and 33.98% for 2θ values 15.97°, 21.96°, 64.80°, and 77.92°, respectively. This gives an average reduction count/intensity of 38.50%, representing the level of breakdown of the cellulose.

4.3.2 Fourier Transform Infrared (FTIR) Spectroscopy

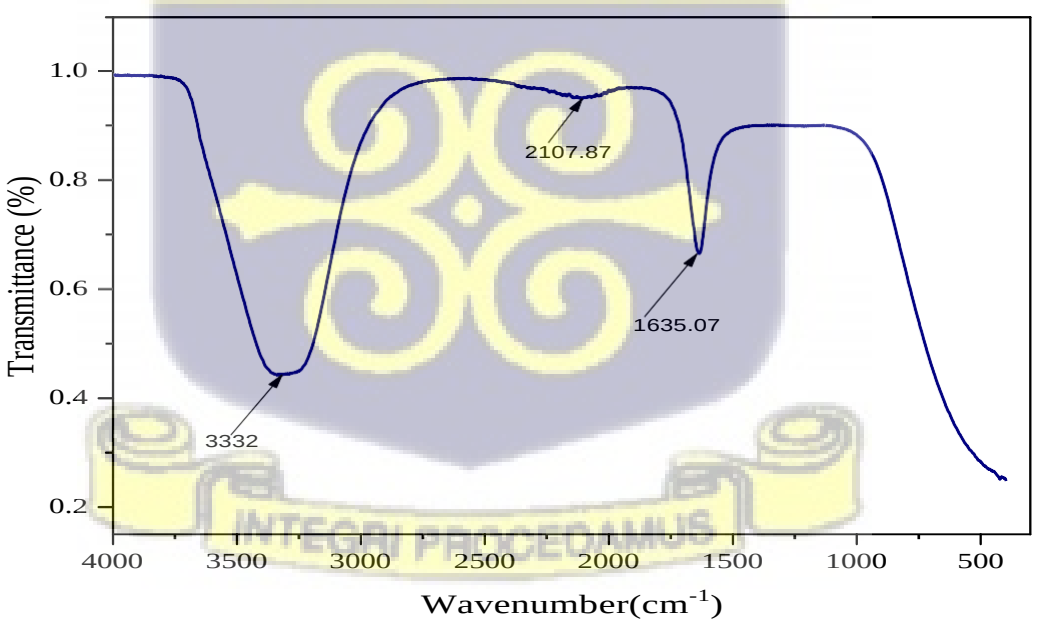

Figure 4.3 FTIR analysis of the distillate

Figure 4.3 clearly shows three peaks at 3332.00 cm^{-1}, 2107.87cm^{-1}, and 1635.04cm^{-1} with transmittances at 0.44%, 0.95%, and 0.67%, respectively. From data, the O•H functional group in alcohols and carboxylic acids shows frequency of absorption in the ranges (3200-3700) cm^{-1} and (2500-3300) cm^{-1}, respectively. In both alcohols and carboxylic acids, the presence of the O-H group shows broad peak due to extensive hydrogen bond. Since there is an overlap of the ranges, an exclusive statement cannot be made about the source of the absorption at 3332 cm^{-1}, it probably may be coming from alcohol source, carboxylic source or both. The C=O frequency of absorption range for carbonyl compounds: aldehydes, ketones and carboxylic acids is 1625-1750 cm^{-1}. Fig.4.3 shows frequency of absorption at 1635.04 cm^{-1} which falls within the given range of 1625-1750 cm^{-1} for aldehydes, ketones and carboxylic acids. Therefore, it is obvious that the frequency of absorption at 1635.04 cm^{-1} in the figure 4.3 is from a carbonyl source which could be an aldehyde, a ketone, and/or a carboxylic acid. Lastly, data shows that the frequency of absorption range 1650-2000 cm^{-1} is C - H bending of an aromatic compound with a weak absorbance. Therefore, the weak and broad absorption at 2107.87 cm^{-1} in Fig. 4.3 may be coming from an aromatic source. It is important to mention that organic acids, aromatic organic compounds, (i.e., phenolics) mostly from the lignin component of the biomass, furfural, and 5-hydroxymethylfurfural, (5-HMF) that may be formed from ~~a~~ five-member carbon sugars such as xylose, arabinose are formed from the degradation of the sugars during hydrolysis. Thus, further characterisation is required to qualitatively identify some of these chemical components in the distillate.

4.3.3 Gas Chromatography-mass Spectrometry Analysis (GC-MS)

The distillate was subjected to gas chromatography-mass spectrometry analysis to identify the chemical impurities present in the sample, and to quantify these impurities by means of peak height in a chromatogram plot. Fig.4.4 is a chromatogram plot of percent peak height versus retention time in minutes.

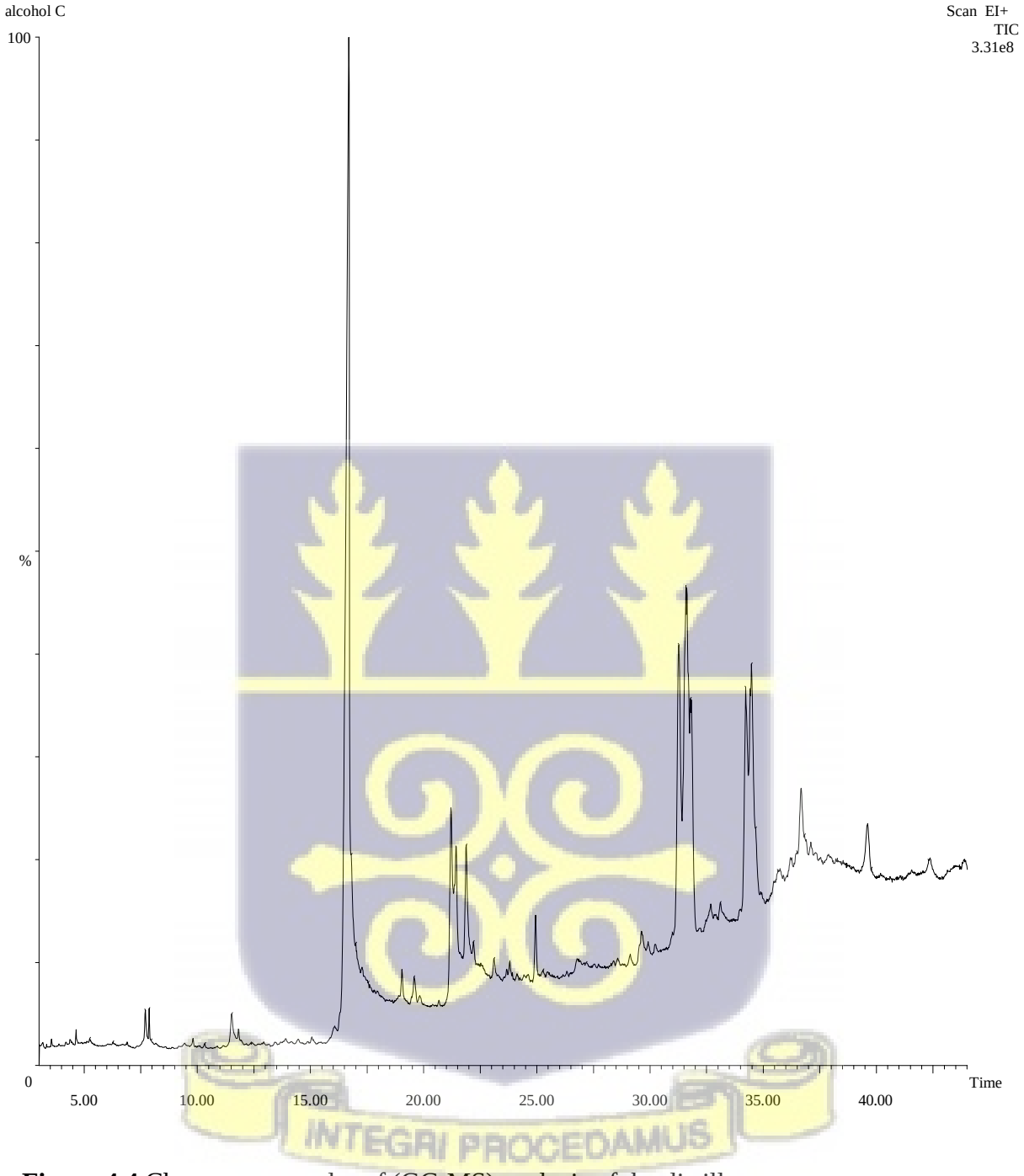

Figure 4.4 Chromatogram plot of (GC-MS) analysis of the distillate

The Figure 4.4 is a chromatogram plot of peak height (%) versus retention time (RT) of GC-MS analysis of the distillate. This analysis provides qualitative and quantitative information regarding the presence of the chemical impurities in the distillate, and the peak height measures the percent composition of these chemical impurities. The elution of ethanol was suppressed within the first 5 mins since the target of this analysis was to identify other chemical components present in the distillate. Fifteen peaks of significant heights are presented in this analysis. The nom peak, the tallest peak based on which percent values of the other peaks are calculated appeared on the 16.695th minute, and is made of n-Hexadecanoic acid, which does not appear anywhere as eluent in the other peaks. The next highest peak appeared on the 31.583st minute with 55.7 % nom, which comprises the eluents, dodecanoic acid, 1,2,3-propanetriyl ester, lauric anhydride, and ethenyl ester. The peak with the least nom value of 3.37% appeared on the 24.946th min and is composed of hexadecanoic acid, 1-(hydroxymethly)-1,2-ethanediyl ester, glycerol, 1-palmitate, ascorbic acid, and 2,6-dihexadecanoate. Dodecanoic acid appeared five retention times (RTs), representing 33.3 % of the fifteen analysed peaks. A total of 41 different compounds are identified in the distillate which comprises inorganic compounds like silane, organic acids, organic salts, esters and so on.

Table 4.2 Retention time, percentage area, nom percent, and names of eluents.

No	RT	Area%	Nom%	Name
1	16.695	26.297	100.00	n-Hexadecanoic acid
2	21.205	3.095	11.81	Oleic acid, 9-octadecenoic acid, cis-vaccenic acid
3	21.425	2.675	10.21	Ethyl oleate
4	21.884	1.996	7.62	Octadecanoic acid
5	24.946	0.884	3.37	Hexadecanoic acid, 1-(hydroxymethly)-1,2-ethanediyl ester, glycerol 1-palmitate, Ascobic acid, 2,6-

				dihexadecanoate
6	31.271	6.853	26.15	Dodecanoic acid, 1,2,3-propanetriyl ester, ethyl ester, lauric anhydride
7	31.583	14.601	55.71	Dodecanoic acid, 1,2,3-propanetriyl ester, lauric anhydride, ethenyl ester
8	34.223	4.792	18.29	N1,N4-Diheptyl-2-(3-methoxy-propylamine)-N1,N4-dimethyl-succinamide, Silane, (1,1-dimethylethyl) diethyl(tetradecyloxy)-, 4H-Benzo[f]pyrrolo[1,2-a][diazepine, 4-cyclohexyl-8,9-diethoxy-5,6-dihydro]-
9	34.480	7.351	28.05	4H-Benzo[f]pyrrolo[1,2-a][diazepine, 4-cyclohexyl-8,9-diethoxy-5,6-dihydro]-, Tridecanoic acid, tert-butyldimethylsilyl ester, 1-Trimethylsilyloxytetradecane
10	35.745	1.668	6.37	Dodecyl cis-9,10-epoxyoctadecanoate, dodecanoic acid, 1-(hydromethyl)-1,2-ethanediyl ester, oleic acid, 3-(octadecyloxy)propyl ester
11	36.204	1.308	4.99	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, 1,2,3-propanetriyl ester, 2-hydroxy-1-(hydroxymethyl) ethyl ester
12	36.680	4.513	17.22	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, 1,2,3-propanetriyl ester, 3,5,9-Trioxa-4-phosphaheneicosan-1-aminium, 4-hydroxy-N,N,N-trimethyl-10-oxo-7-[(1-oxododecy…
13	37.102	2.680	10.23	Phorbol 12,13-dihexanoate, Phorbol 12,13,20-triacetate, decanoic acid, 1,1a,1b,4,4a,5,7a,7b,8,9,-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetram…, phorbol12,13,20-triacetate

14	37.909	1.440	5.49	Ethyl iso-allocholate, propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)- Psi., psi.-carotene 1,1`,2,2`tetrahydro-1,1`-dimethoxy-
15	39.614	1.437	5.48	Dodecanoic acid, 3-dodecanoyloxy-propyl ester, octadecanoic acid, decyl ester, fumaric acid, dodecyl octyl ester

4.3.4 Ultraviolet-visible Light (UV-Vis) Spectroscopy

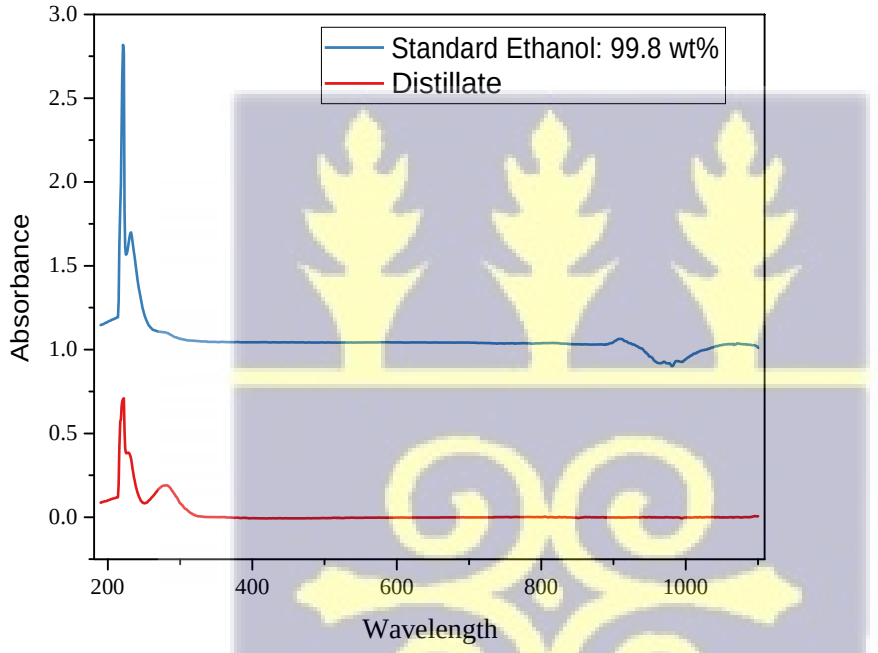

Figure 4.5 (a) Comparison of the spectrum of standard grade ethanol at 99.8 wt% purity to the spectrum of the distillate.

Fig.4.5 (a) is UV-vis spectroscopy analysis of standard grade ethanol at 99.8 wt% purity spectrum and the distillate of this research. UV-Vis spectroscopy characterization is used to ascertain the presence or otherwise of other chemical impurities by comparing the spectrum of the standard ethanol to that of the distillate. GC-MS spectroscopy analysis shown in figure 4.4 did indicate a substantial number of chemical impurities in the distillate that could not be

shown in the UV- vis spectrum, most probably due to their low concentrations. Thus, it serves as a qualitative test for the distillate. There is some semblance in the two graphs, especially in the wavelength range of 220-340 nm. Two peaks are clearly shown on both graphs. The standard ethanol graph (i.e., red graph) shows two peaks at 220 nm and 233 nm and absorbance of 1.78 and 0.64, respectively. The corresponding peaks of the distillate show similar characteristics with wavelength 220 nm and 280 nm and absorbance of 0.69 and 0.19, respectively. The stretching of the latter at 280 nm as compared to 233 nm of the former might probably be as result of chemical impurities in the distillate. The absorbance further braced this point as the standard ethanol graph shows absorbance of 1.73 and 0.64 for its two peaks as compared to the absorbance of 0.69 and 0.19 for the two peaks of the distillate.

Further UV-vis spectroscopy analysis was done to determine the ethanol concentration in the distillate. Table 4.3 shows ethanol concentrations that were prepared and their respective absorbance measured at maximum ethanol absorption wavelength of 220 nm.

Table 4.3 shows nine ethanol concentrations that were prepared for the construction of the calibration curve**.**

Ethanol Conc. (V/V)	5	**10**	**15**	**20**	**25**	**30**	40	**50**	**100**
Absorbance (a.u)	0.256	0.440	0.565	0.695	0.800	0.899	1.100	1.400	2.080

The results in Table 4.3 agree with Beer Lambert's Law as concentration varies linearly with absorbance.

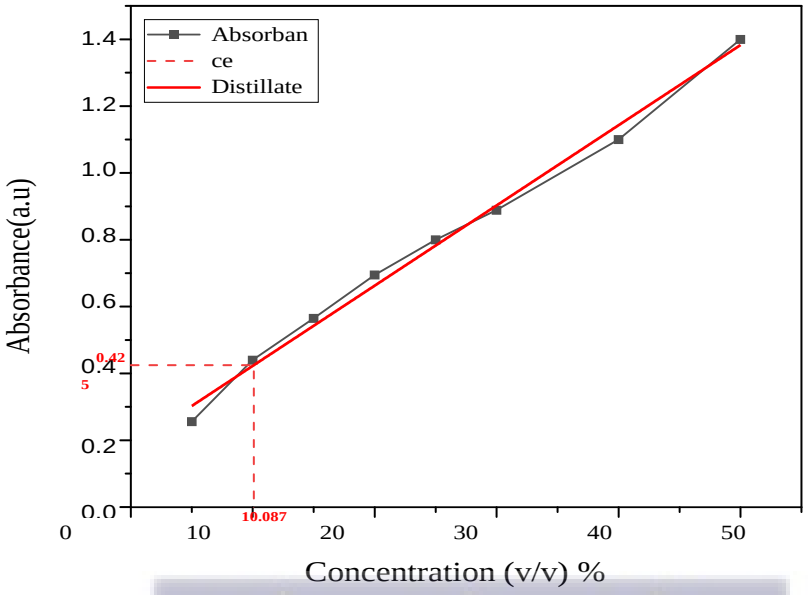

Figure 4.5 (b) Calibration curve constructed from prepared ethanol concentrations and their respective absorbance.

The measured absorbance of the distillate, 0.425 is extrapolated on the graph shown in red broken lines gives a concentration value of 10.0875 % (v/v), which is equivalent to 0.884 g/L, (or 1.92 x10^{-2} mol/L). Since absorption is proportional to concentration, the gradient of the curve must be equal to the molar absorption coefficient, Ɛ, for a curvette of length 1 cm. Thus, 0.425 absorbance of the distillate and a concentration of 1.92 x 10^{-2} mol/L, gives the molar absorption coefficient, Ɛ, 22.14 L/mol.cm. The curve has R-square, coefficient of dispersion, (COD), 0.99296, which shows the extent of linearity between absorbance and concentration.

It is imperative to make a comparison of the 10.0875 % (v/v) obtained in this work to a couple of works published in this field of study. In their work, 'Bioethanol production from coconut fibre using alkaline and acid hydrolysis', Jannah et al reported an ethanol concentration of 5.9 % (v/v), using the microbial strains S. *cerevisiae* at a pH of 4.5. Soares et

al reported an ethanol concentration of 4.33 % (v/v) using the SHF strategy and the microbial strains S. *cerevisae* at a pH of 5.5. In their extraction of bioethanol from corn stover, Saha et al used SO_2- catalyzed steam explosion and using baker's yeast they reported an ethanol concentration of 0.29 g/L an equivalent of 3.31 % (v/v) at a pH of 5.5.

CHAPTER FIVE

5.0 Conclusion and Recommendation

5.1Conclusion

Extraction of bioethanol fro m corn husk optimizes the treatment conditions, feed acid concentration and post-hydrolysis temperature, and employ a more subtle approach in maximizing the sugar yield while using a relatively small amount of the acid. The methodology involves a physicochemical pre-treatment of the corn husk with concentrated sulphuric acid, and heating below 60 ^{o}C for 1 hr. This is followed by diluting the acid and heating the mixture above 60 ^{o}C for 1 hr. In optimizing the process, a feed acid concentration of 30 wt% and post-hydrolysis temperature 80 ^{o}C gave the best yield of the sugar, 33^{o} Brix.

The microbial strain, *Sacchoramyces cerevisiae* was the fermentive microorganism. The period of fermentation was 14 days. Ethanol in the fermentation broth was estimated to be 2.9 %. X-Ray powder Diffraction (XRD) patterns obtained from the corn husk and residual solid residue show a decrease in the intensity/counts by 38.50 % showing the level of breakdown of the crystalline cellulosic mass.

Fourier Transform Infrared (FTIR) spectroscopy analysis shows the presence of O-H functional group, carbonyl C=O functional group, and C-H bending of an aromatic compound.

GC-MS analysis identified n-Hexadecanoic acid as the chemical component with the largest amount in the distillate. The analysis identified a total of 41 different chemical components in the distillate.

UV-vis spectroscopy for an identity test shows semblance of the spectrum of standard grade ethanol at 99.8 wt% purity to the spectrum of the distillate with both absorbing in range 220-340 nm.

Further UV-vis spectroscopy analysis on the distillate quantified the ethanol concentration at10.0875 v/v %, an equivalent of 0.884 g/L, (or 1.92 $x10^{-2}$ mol/L).

5.2 Recommendations

Future perspective studies in this area maybe interested in investigating the following areas:

1. Recovering the acid catalyst used during hydrolysis and testing its quality and the prospects of its re-usage

2. Secondly, the sugars that leached during hydrolysis should be qualitatively identified and quantified.

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